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In re Application of:

Nelson et al.

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Application No. 09/242,202

Examiner: Li, Qian J.

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For: A NOVEL VECTOR FOR POLYNUCLEOTIDE VACCINES

PENDING CLAIMS AFTER AMENDMENTS MADE IN RESPONSE TO OFFICE ACTION DATED FEBRUARY 13, 2002

1. A humanized polynucleotide vector comprising:

a human derived promoter or mammalian homolog thereof, either one of which is functional in a target tissue or target cells, said promoter operably linked to a sequence acceptance site, which directionally accepts cDNA derived from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding vector-derived polypeptides, wherein said vector lacks an antibiotic resistance encoding nucleic acid sequence.

2. The humanized polynucleotide vector according to claim 1 wherein the target cells are selected from the group consisting of myocytes and professional antigen presenting cells.

3. The humanized polynucleotide vector according to claim 1 or 2 wherein the target cells or target tissue are human.

4. The humanized polynucleotide vector according to claim 1 wherein the human derived promoter is a RANTES promoter or portion thereof.

5. The humanized polynucleotide vector according to claim 4 wherein the promoter has approximately 440 base pairs.

6. The humanized polynucleotide vector according to claim 4 or 5 wherein the portion corresponds to a region spanning the NCO site through the KpnI site of the genomic RANTES promoter.

7. The humanized polynucleotide vector according to claim 1 further comprising an origin for replication and growth and a nucleic acid sequence which allows for selection of recombinant plasmids.

8. The humanized polynucleotide vector according to claim 7 wherein the origin for replication is colE1 or functional portion thereof.

9. The humanized polynucleotide vector according to claim 7 wherein the origin for replication comprises a 635 base pair region of the colE1 origin of replication.

10. The humanized polynucleotide vector according to claim 1 further comprising a human-derived 3' splice sequence and a human-derived poly A sequence, both sequences located downstream of the sequence acceptance site.

11. The humanized polynucleotide vector according to claim 10 wherein the human derived 3' splice and poly A sequence are derived from human growth hormone.

12. A polynucleotide vector according to claim 1 in which the sequence acceptance site is a 5' sequence site having the nucleotide sequence GCCACCATGGCC on the positive strand.

13. A polynucleotide vector comprising SEQ ID No 16, SEQ ID No 27 or SEQ ID No 28.

14. A polynucleotide vector contained within a host cell deposited with the ATCC designation 98400 or ATCC designation 98401.

15. A polynucleotide vector according to claim 1 further comprising cDNA derived from rtPCR cloning, and an optional internal ribosomal entry site, said cDNA integrated into said sequence acceptance site, said cDNA comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof alone or in combination with a nucleotide sequence encoding a cytokine or chemokine.

16. A composition for inducing an immune response against at least one target antigen or antigenic epitope comprising a vector comprising a human derived promoter or mammalian homolog thereof, either one of which is functional in a mammalian target tissue or mammalian target cell, said promoter operably linked to a sequence acceptance site, which directionally accepts cDNA derived from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, an optional internal ribosomal entry site, and cDNA derived from said rtPCR cloning, said cDNA integrated into said sequence acceptance site, said cDNA comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof, wherein said vector induces an immune response to said antigen or antigenic epitope thereof, and said vector lacking nucleic acid sequences encoding vector-derived polypeptides, wherein said vector lacks an antibiotic resistance encoding nucleic acid sequence.

17. A composition for inducing an immune response according to claim 16 wherein the target antigen is a product of a tumor associated genetic derangement.

18. A composition for inducing an immune response according to claim 16 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen, or parasitic antigen.

19. The composition for inducing an immune response according to claim 16, wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAP, MEN-1, ERB-B1 and combinations thereof.

20. A composition for inducing an immune response according to claim 16 further comprising an additional cDNA derived from rtPCR, comprising a nucleic acid sequence encoding a cytokine or chemokine.

21. A composition for inducing an immune response according to claim 20 wherein the cytokine is selected from the group consisting of interleukin 2, interleukin 3, interleukin 4, interleukin 7, interleukin 8, interleukin 12, interleukin 15, GM-CSF, tumor necrosis factor, and interferon.

22. A composition for inducing an immune response according to claim 20 wherein the chemokine is selected from the group consisting of RANTES, MCP, MIP-Eα, MIP-1β, defensins, IP-10 and combinations thereof.

23. A method for expressing at least one target antigen or antigenic epitope thereof in cells comprising:

introducing a humanized polynucleotide vector into said cells, under conditions for expression of the target antigen or antigenic epitope thereof, said vector comprising:

a human derived promoter or mammalian homolog thereof, which is functional in said cells, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA derived from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease and,

cDNA derived from rtPCR, and an optional internal ribosomal entry site, said cDNA integrated into said sequence acceptance site, said cDNA comprising a nucleic acid sequence encoding at least one target antigen or antigenic epitope thereof, and said vector lacking nucleic acid sequences encoding vector-derived polypeptides, wherein said vector lacks an antibiotic resistance encoding nucleic acid sequence.

24. The method of claim 23 wherein the cells are selected from the group consisting of myocytes and professional antigen presenting cells.

25. The method of claim 23 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen, or parasitic antigen.

26. The method of claim 25 wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC,

Myb, OB-l, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 or combinations thereof.

27. A composition comprising at least one polynucleotide vector according to claims 1, 2, 4, 5, 7 or 8-12 and a pharmaceutically acceptable carrier.

28. A composition comprising a composition for inducing an immune response according to claims 16-21 or 22 and a pharmaceutically acceptable carrier.

29. A kit comprising the polynucleotide vector according to claims 1, 2, 4, 5, or 7-15.

30. A kit comprising the composition according to claims 16-22 or 22.

31. A kit according to claim 30, further comprising an expression enhancing agent.

32. The kit according to claim 31 wherein the expression enhancing agent is a mycotoxic agent.

33. The kit according to claim 32 wherein the mycotoxic agent is bupivacaine-HCl and dextrose.

34. (Canceled)

35. (Canceled)

36. A method of stimulating a specific immune response to at least one target antigen or antigenic epitope thereof in a mammal comprising: administration of an effective amount of a composition according to claims 16-21 or 22 into the mammal, said amount elicits the specific immune response to the target antigen or epitope thereof.

37. The method according to claim 36, wherein a site of administration is muscle or skin.

38. The method according to claim 36 further comprising administration of an effective amount of an expression enhancing agent prior to administration of said composition.

39. The method according to claim 38 wherein the expression enhancing agent is a mycotoxic agent.

40. The method according to claim 39 wherein the mycotoxic agent is bupivacaine-HCl or dextrose.

41. The method according to claim 36-39 or 40 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen or parasitic antigen.

42. The method according to claim 41 wherein the tumor antigen is selected from the group consisting of p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-l, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 and combinations thereof.

43. The method according to claim 42 wherein the method generates antigen specific cytotoxic lymphocytes to the tumor antigen or antigenic epitopes thereof.

44. A method of making a humanized polynucleotide vector comprising:

operably linking a human derived promoter or mammalian homolog thereof, either of which is functional in a target tissue or target cells, to a sequence acceptance site, said site directionally accepts cDNA derived from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding a vector-derived polypeptide, wherein said vector lacks an antibiotic resistance encoding nucleic acid sequence.

45. (Canceled)

46. (Canceled)

47. (Canceled)

48. (Canceled)

49. (Canceled)

50. (Canceled)

51. (Canceled)

60. The humanized polynucleotide vector according to claims 1, 2, 4, 5 or 7-15, wherein the recognition sequence is recognized by Bg1I restriction endonuclease.

61. The humanized polynucleotide vector according to claim 7, wherein the nucleic acid sequence which allows for selection is a suppressor tRNA gene, a synthetic SupF complementation tRNA gene, or functional derivatives thereof.

62. The humanized polynucleotide vector according to claim 61, wherein the nucleic acid sequence is selected from the group consisting of SupE, SupP, SupD, SupU, SupF, SupZ, glyT, glyU, SerP, $psul^{+}$, $psu2^{+}$-C34, $psu3^{+}$AM and $psu3^{-}$OC.

63. A polynucleotide vector according to claims 1, 2, 4, 5 or 7-l 1, wherein a 3' sequence acceptance site reads on the position strand as GCCTTAAGGGC.

64. The humanized polynucleotide vector according to claims 1, 2, 4, 5 or 7-11, wherein the sequence acceptance site comprises the nucleotide sequence as depicted in Figure 2.

65. The method according to any of claims 23-25 or 26 wherein the method is *ex vivo.*

66. A humanized polynucleotide vector comprising:

a human derived promoter or mammalian homolog thereof chosen from the group consisting essentially of a human derived RANTES promoter, a truncated RANTES promoter, a truncated RANTES promoter of 249 base pairs, a truncated RANTES promoter of 440 base pairs, a truncated RANTES promoter of 900 base pairs or a truncated RANTES promoter as described in GenBank Accession No. S64885, which is

functional in a target tissue or target cells, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA derived from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding vector-derived polypeptides wherein, said vector lacks an antibiotic resistance encoding nucleic acid sequence.

67. The humanized polynucleotide vector according to claim 66 wherein the target cells are selected from the group consisting of myocytes and professional antigen presenting cells.

68. The humanized polynucleotide vector according to claim 66 or 67 wherein the target cells or target tissue are human.

69. The humanized polynucleotide vector according to claim 66 further comprising an origin for replication and growth and a nucleic acid sequence which allows for selection of recombinant plasmids.

70. The humanized polynucleotide vector according to claim 69 wherein the origin for replication is colE1 or functional portion thereof.

71. The humanized polynucleotide vector according to claim 69 wherein the origin for replication comprises a 635 base pair region of the colE1 origin of replication.

72. The humanized polynucleotide vector according to claim 66 further comprising a human-derived 3' splice sequence and a human-derived poly A sequence, both sequences located downstream of the sequence acceptance site.

73. The humanized polynucleotide vector according to claim 72 wherein the human derived 3' splice and poly A sequence are derived from human growth hormone.

74. A polynucleotide vector according to claim 66 wherein a 5' sequence acceptance site reads on the positive strand as GCCACCATGGCC.

75. A polynucleotide vector comprising SEQ ID No 16, SEQ ID No 27 or SEQ ID No 28.

76. A polynucleotide vector contained within a host cell deposited with the ATCC designation 98400 or ATCC designation 98401.

77. A polynucleotide vector according to claim 66 further comprising cDNA derived from rtPCR cloning, and an optional internal ribosomal entry site, said cDNA integrated into said sequence acceptance site, said cDNA comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof alone or in combination with a nucleotide sequence encoding a cytokine or chemokine.

78. A composition for inducing an immune response against at least one target antigen or antigenic epitope comprising a vector comprising a human derived promoter or mammalian homolog thereof chosen from the group consisting essentially of a human derived RANTES promoter, a truncated RANTES promoter, a truncated RANTES promoter of 249 base pairs, a truncated RANTES promoter of 440 base pairs, a truncated RANTES promoter of 900 base pairs or a truncated RANTES promoter as described in GenBank Accession No. S64885, which is functional in a mammalian target tissue or mammalian target cell, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA derived from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, an optional internal ribosomal entry site, and cDNA derived from said rtPCR cloning, said cDNA integrated into said sequence acceptance site, said cDNA comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof, wherein said vector induces an immune response to said antigen or antigenic epitope thereof, and said vector lacking nucleic acid sequences encoding vector-derived polypeptides wherein, said vector lacks an antibiotic resistance encoding nucleic acid sequence.

79. A composition for inducing an immune response according to claim 78 wherein the target antigen is a product of a tumor associated genetic derangement.

80. A composition for inducing an immune response according to claim 78 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen, or parasitic antigen.

81. A composition for inducing an immune response according to claim 78, wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-l, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAP, MEN-1, ERB-B1 and combinations thereof.

82. A composition for inducing an immune response according to claim 78 further comprising an additional cDNA derived from rtPCR, comprising a nucleic acid sequence encoding a cytokine or chemokine.

83. A composition for inducing an immune response according to claim 82 wherein the cytokine is selected from the group consisting of interleukin 2, interleukin 3, interleukin 4, interleukin 7, interleukin 8, interleukin 12, interleukin 15, GM-CSF, tumor necrosis factor, interferon.

84. A composition for inducing an immune response according to claim 82 wherein the chemokine is selected from the group consisting of RANTES, MCP, MIP-Eα, MIP-1β, defensins, IP-10 and combinations thereof.

85. A method for expressing at least one target antigen or antigenic epitope thereof in cells comprising:

introducing a humanized polynucleotide vector into said cells, under conditions for expression of the target antigen or antigenic epitope thereof, said vector comprising:

a human derived promoter or mammalian homolog thereof chosen from the group consisting essentially of a human derived RANTES promoter, a truncated RANTES promoter, a truncated RANTES promoter of 249 base pairs, a truncated RANTES promoter of 440 base pairs, a truncated RANTES promoter of 900 base pairs or a truncated RANTES promoter as described in GenBank Accession No. S64885, which is functional in said cells, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA derived from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease and,

cDNA derived from rtPCR, and an optional internal ribosomal entry site, said cDNA integrated into said sequence acceptance site, said cDNA comprising a nucleic acid sequence encoding at least one target antigen or antigenic epitope thereof,

and said vector lacking nucleic acid sequences encoding vector-derived polypeptides, wherein said vector lacks an antibiotic resistance encoding nucleic acid sequence.

86. The method of claim 85 wherein the cells are selected from the group consisting of myocytes and professional antigen presenting cells.

87. The method of claim 85 wherein the target antigen is a tumor antigen bacterial antigen, viral antigen, or parasitic antigen.

88. The method of claim 87 wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-l, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 or combinations thereof.

89. A composition comprising at least one polynucleotide vector according to claims 66, 67, 69 or 70-74 and a pharmaceutically acceptable carrier.

90. A composition comprising a composition for inducing an immune response according to claims 78-84 and a pharmaceutically acceptable carrier.

91. A kit comprising the polynucleotide vector according to claims 66, 67 or 69-77.

92. A kit comprising the composition according to claims 78-84.

93. A kit according to claim 92, further comprising an expression enhancing agent.

94. The kit according to claim 93 wherein the expression enhancing agent is a mycotoxic agent.

95. The kit according to claim 94 wherein the mycotoxic agent is bupivacaine-HCl and dextrose.

96. A method of stimulating a specific immune response to at least one target antigen or antigenic epitope thereof in a mammal comprising: administration of an

effective amount of a composition according to claims 78-84 into the mammal, said amount elicits the specific immune response to the target antigen or epitope thereof.

97. The method according to claim 96, wherein a site of administration is muscle or skin.

98. The method according to claim 96 further comprising administration of effective amount of an expression enhancing agent prior to administration of said composition.

99. The method according to claim 98 wherein the expression enhancing agent is a mycotoxic agent.

100. The method according to claim 99 wherein the mycotoxic agent is bupivacaine-HCl or dextrose.

101. The method according to claims 96-100 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen or parasitic antigen.

102. The method according to claim 101 wherein the tumor antigen is selected from the group consisting of p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-l, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 and combinations thereof.

103. The method according to claim 102 wherein the method generates antigen specific cytotoxic lymphocytes to the tumor antigen or antigenic epitopes thereof.

104. A method of making a humanized polynucleotide vector comprising:

operably linking a human derived promoter or mammalian homolog thereof chosen from the group consisting essentially of a human derived RANTES promoter, a truncated RANTES promoter, a truncated RANTES promoter of 249 base pairs, a truncated RANTES promoter of 440 base pairs, a truncated RANTES promoter of 900 base pairs or a truncated RANTES promoter as described in GenBank Accession No. S64885, which is functional in a target tissue or target cells to a sequence acceptance site, said site directionally accepts cDNA derived from rtPCR cloning via unique sites within

an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding a vector-derived polypeptide wherein, said vector lacks an antibiotic resistance encoding nucleic acid sequence.

105. The humanized polynucleotide vector according to claims 66,67 or 69-77, wherein the recognition sequence is recognized by Bg1I restriction endonuclease.

106. The humanized polynucleotide vector according to claim 69, wherein the nucleic acid sequence which allows for selection is a suppressor tRNA gene, a synthetic SupF complementation tRNA gene, or functional derivatives thereof.

107. The humanized polynucleotide vector according to claim 106, wherein the nucleic acid sequence is selected from the group consisting of SupE, SupP, SupD, SupU, SupF, SupZ, glyT, glyU, SerP, $psul^{+}$, $psu2^{+}$-C34, $psu3^{+}$AM and $psu3^{-}$OC.

108. A polynucleotide vector according to claims 66, 67 or 69-73, wherein the sequence acceptance site is a 3' sequence having the nucleotide sequence GCCTTAAGGGC on the positive strand.

109. The humanized polynucleotide vector according to claims 66, 67 or 69-73, wherein the sequence acceptance site comprises the nucleotide sequence as depicted in Figure 2.

110. The method according to any of claims 85-88 wherein the method is *ex vivo*.